

HSPD1 is not a major susceptibility gene for rheumatoid arthritis in the French Caucasian population

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Received: 9 July 2007 / Accepted: 18 September 2007 / Published online: 11 October 2007
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Abstract The heat shock 60-kDa protein 1 (HSP60) is involved in immune and inflammatory reactions, which are hallmarks of rheumatoid arthritis (RA). HSP60 is encoded by the *HSPD1* gene located on 2q33, one of the suggested RA susceptibility loci in the French Caucasian population. Our aim was to test whether *HSPD1* is a major susceptibility gene by studying families from the French Caucasian population. Three single nucleotide polymorphisms (SNPs) were studied in 100 RA trio families, and 100 other families were used for replication. Genetic analyses were performed by comparing allelic frequencies, by applying the transmission disequilibrium test, and by assessing the genotype relative risk. We observed a significant RA association for the *C/C* genotype of rs2340690 in the first sample. However, this association was not confirmed when the second sample was added. The two other SNPs and the

haplotype analysis did not give any significant results. We conclude that *HSPD1* is not a major RA susceptibility gene in the French Caucasian population.

Keywords *HSPD1* · Rheumatoid arthritis · Candidate gene · Heat shock protein · Chaperonin

Introduction

Rheumatoid arthritis (RA) is a common human systemic autoimmune disease, for which previous studies have suggested the importance of genetic factors (Seldin et al. 1999). Two susceptibility genes have been established so far (and are confirmed by the results from the sample sets of the present study), *HLA-DRB1* and *PTPN22*, which account for 19 and 1% of the familial aggregation respectively (Tezenas du Montcel et al. 2005; Michou et al. 2007). A genome scan suggested 19 non-*HLA* susceptibility loci in a French Caucasian population (Osorio et al. 2004). *HSPD1* is located in one of these regions (2q33), and encodes for a member of the chaperonin family (heat shock 60-kDa protein 1, HSP60). Heat shock proteins constitute a family of proteins involved in cell homeostasis, immune and inflammatory reactions. They can regulate gene expression, cell proliferation, and death. HSP60 is a mitochondrial chaperonin, highly preserved during evolution, and responsible for protein folding (Cappello et al. 2004). Homozygous *Drosophila melanogaster HSPD1* homolog *–/–* mutants die early on during embryogenesis (Perezgasga et al. 1999). In human cells, knockdown of the *HSPD1* gene compromises the folding of the mitochondrial matrix enzymes, indicating that HSP60 plays an essential role (Corydon et al. 2005). Furthermore, HSP60 can downregulate adaptive immune responses and is involved

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in apoptosis in patients with systemic autoimmune diseases (Zanin-Zhorov et al. 2006; Jamin et al. 2005). Interestingly, DNA vaccination with the *HSPD1* gene can inhibit adjuvant arthritis in Lewis rats (Quintana et al. 2003).

HSPD1 is thus a positional and functional RA candidate gene. The aim of this study was to test whether *HSPD1* is a major susceptibility gene by analyzing families from the French Caucasian population.

Material and methods

Rheumatoid arthritis families were recruited through a media campaign, and then individuals who fulfilled the 1987 American College of Rheumatology criteria were selected (Arnett et al. 1988). All subjects provided informed consent, and an ethics committee (Kremlin-Bicêtre, France) approved the study. The two samples included DNA from 100 unrelated French Caucasian trio families (a patient and both parents) along with four grandparents of French Caucasian origin (Table 1).

DNA was isolated and purified from whole blood according to standard protocols (Sambrook et al. 1989). The single nucleotide polymorphisms (SNPs) were selected with a required minor allele frequency of >18% among the European Caucasian population and $r^2 > 0.8$ using the QuickSNP web server (<http://bioinformoodics.jhmi.edu/quickSNP.pl>).

Genotyping

Genotyping of rs2340690 was performed by polymerase chain reaction followed by the restriction fragment length polymorphism method (Botstein et al. 1980). The designed primers are available on request. The resulting 434-bp fragment was digested with *AluI*, generating three

fragments for the *C* allele (68, 103, 263 bp), and two for the *T* allele (103, 331 bp, the 103 bp being an internal control for restriction). Allelic discrimination assays (assay C 8744787 10, C 16261693 10; Applied Biosystems, Foster City, CA, USA) were used to genotype rs788016 and rs2605039, respectively, following the manufacturer's protocol. Genotypes were assessed blindly by two investigators (LJ-CP), and 10% of the samples (chosen randomly) were regenotyped for quality control.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was checked for with virtual controls consisting of parental alleles untransmitted to RA index cases. Association and linkage were examined using three methods. Affected family-based controls (AF-BAC) were used to compare transmitted and untransmitted allelic frequencies. The transmission disequilibrium test (TDT) was used to detect linkage through preferential transmission of one allele to the affected subjects. The genotype relative risk (GRR) was calculated to compare the genotypic distributions in patients and controls (Spielman et al. 1993; Thomson 1995; Lathrop 1983). Significance of the *P* value was assessed at the 5% level, which led to a replication test in 100 other families (sample 2) if at least one test was significant. In case of a positive replication, 265 European Caucasian families were available.

Power calculation

Using the European population minor allele frequencies of 20, 43 and 25% for rs2340690, rs788016 and rs2605039, respectively, with a sample size of 100 patients and 100 controls, as previously described (Garnier et al. 2006), we had 80% power to detect an association ($P < 0.05$) when the difference in allelic frequencies between patients and controls was at least 10.7, 12.4 and 11.4%, respectively.

Results

There are three SNPs that tag the three different LD blocks that exist in *HSPD1* (without containing the other genes, rs2340690, rs788016 and rs2605039). In a recent large-scale genome-wide association study in the British population, one of them (rs788016) and one SNP in LD with rs2605039 (rs8539) were tested without significant results being obtained (Wellcome Trust Case Control Consortium 2007). As we performed a different approach (family-based) in a different population (French Caucasian), we tested these three SNPs. The observed genotype

Table 1 Characteristics of the rheumatoid arthritis index cases

	Sample 1 (<i>n</i> = 100)	Sample 2 (<i>n</i> = 100)
Females (%)	87	90
Mean age at disease onset (years)	32 (±10)	31 (±6)
Mean disease duration (years)	18 (±7)	16 (±8)
Patients with bone erosion (%)	90	79
Patients seropositive for rheumatoid factor (%)	81	76
Patients carrying at least one <i>HLA-DRB1</i> shared epitope allele* (%)	78	80

DRB10101, *DRB1**0102, *DRB1**0401, *DRB1**0404, *DRB1**0405, *DRB1**0408, *DRB1**1001

n is the number of cases

frequencies were in accordance with the HWE in controls. We observed a trend for overtransmission of the *C* allele with the *C/C* genotype significantly more frequent in cases than in controls for rs2340690 ($P = 0.04$, Table 2). This association led to a replication test in sample 2 with the hypothesis of a *C/C* genotype association with RA. This finding was not confirmed, and the allelic frequencies were nearly identical between cases and controls. The combined analysis of the two clinically identical samples (200 families) did not yield a significant result (Table 2).

Neither significant association nor linkage to RA was found for rs788016 and rs2605039 (Table 3), in agreement with the British study (Wellcome Trust Case Control Consortium 2007). The absence of linkage disequilibrium (LD) between the three SNPs was confirmed, and the results of the haplotype TDT analysis were not significant (data not shown).

When stratifying the sample for families with index presenting at least one *PTPN22-620W* allele or the *HLA-DRB1* allele shared epitope status, no correlation with the *HSPD1* genotypes was observed (data not shown).

Discussion

We studied *HSPD1*, a positional and functional RA candidate gene, since we hypothesized that it is a major

Table 2 Affected family-based controls (AFBAC), transmission disequilibrium test (TDT), and genotype relative risk (GRR) analyses for rs2340690

Allele and sample		AFBAC			TDT		
		Cases	Controls	<i>P</i>	% of trans	<i>n</i>	<i>P</i>
C, sample 1		0.24	0.17	0.09	60	65	0.1
C, sample 2		0.21	0.20	0.7	52.3	63	0.7
C, sample 1 + 2		0.23	0.18	0.14	56.2	128	0.15
Genotype	Cases	Controls	<i>P</i>				
GRR sample 1							
<i>C/C</i>	7	3	0.04 (<i>C/C</i> vs. others)				
<i>C/T</i>	32	27					
<i>T/T</i>	55	64					
GRR sample 2							
<i>C/C</i>	3	2	0.9 (<i>C/C</i> vs. others)				
<i>C/T</i>	34	33					
<i>T/T</i>	55	57					
GRR Sample 1 + 2							
<i>C/C</i>	10	5	0.2 (<i>C/C</i> vs. others)				
<i>C/T</i>	66	60					
<i>T/T</i>	110	121					

n is the number of heterozygous parents

Table 3 AFBAC, TDT and GRR analyses for rs788016 and rs2605039

Allele and sample	AFBAC			TDT		
	Cases	Controls	<i>P</i>	% of trans	<i>n</i>	<i>P</i>
<i>Rs788016-A</i> , sample 1	0.49	0.53	0.4	45.9	98	0.4
<i>Rs2605039-G</i> , sample 1	0.26	0.28	0.7	48	77	0.7
Genotype	Cases	Controls	<i>P</i>			
GRR						
<i>Rs788016-A/A</i>	27	28	0.49			
<i>Rs788016-A/G</i>	43	49				
<i>Rs788016-G/G</i>	28	21				
<i>Rs2605039-G/G</i>	8	8	0.9			
<i>Rs2605039-G/T</i>	37	40				
<i>Rs2605039-T/T</i>	55	52				

n is the number of heterozygous parents

susceptibility gene by analyzing families from the French Caucasian population. We observed a significant RA association of the *C/C* genotype for rs2340690 in the first sample, which was not replicated in the second sample.

These results exclude the *rs2340690-C* allele from being a major RA genetic factor, but they cannot totally exclude a minor association. However, it would require 550 trio families with 80% detection power ($P < 0.05$) to find this association, and several thousands to confirm a definitive association ($P < 10^{-6}$).

To test our hypothesis, we studied the three tag SNPs corresponding to our criteria. Thus, we cannot totally exclude the notion that a very minor RA susceptibility allele exists in *HSPD1*.

This 2q3 susceptibility locus contains 101 genes (68 known) including one recently suggested susceptibility gene (Remmers et al. 2007, <http://www.ensembl.org/index.html>). It seems very unlikely that *HSPD1* is involved in this susceptibility locus.

In conclusion, this study provides evidence that *HSPD1* is not a major RA susceptibility gene in the French Caucasian population.

Acknowledgments We thank the families and the following associations: Française-Polyarthritiques, Recherche-Polyarthrite, Polyarctique, Rhumatisme-Travail. Société Française Rhumatologie, Genopole, Université Evry, Shering-Plough, Pfizer, Amgen, Conseil Régional IDF, Conseil Général Essonne, Ministère Recherche-Enseignement Supérieur, Fondation Recherche Médicale, Hôpital Sud Francilien (France), Foundation Sciences-Technology (Portugal).

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